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16. ABSTRACT Low levels of poliovirus 1 were reported to have been isolated from 4 finished water samples collected by an EPA contractor from a water system that used conventional treatment including disinfection. The circumstances surrounding these virus isolations suggest that exogenous contamination of the samples may have occurred. This report discusses these circumstances and also recommends laboratory procedures that would minimize the risk of exogenous contamination of environmental virus samples.		
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**VIRUSES IN FINISHED WATER:
The Occoquan Experience**

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Are there viruses in finished drinking water? This question has been seriously addressed for about 10 to 12 years. The concern over this possibility arises primarily from the circumstances and evidence summarized in Table 1. It was apparent from the beginning of this work that a virus recovery method suitable for studying this question would require the processing of large volumes of water. This is true for several reasons: (1) viruses cannot reproduce outside living host cells, (2) they are inactivated by natural physical, biological and chemical factors at varying rates after leaving the infected host and entering the aquatic environment, (3) the primary source of human enteric viruses in water is sewage, which is diluted many fold as it mixes with the raw water used by a treatment plant, and (4) conventional water treatment procedures further remove or inactivate the viruses. Therefore, viruses would not be expected to be present in finished waters in large numbers.

In the last four to five years, virus recovery methods have emerged that permit the sampling of as much as 19,000 liters (5,000 gal) of water^{1,2}. The methods have evolved to basically one technique in which the water sample is passed through a virus adsorbing filter under defined conditions and eluted with a small volume of fluid that is then further reduced in volume via a reconcentration step. This basic procedure has found wide acceptance by investigators working in environmental virology. A method utilizing this technique was included in the 14th Edition of Standard Methods as a tentative standard procedure for finished waters³.

The virology program at the Environmental Research Center in Cincinnati has used this basic procedure to test 380 to 1900-liter (100 to 500 gal.) samples of finished water from 35 treatment systems in several states. In the last three years, we have collected and analyzed approx. 100 samples from these sites and have not isolated a single virus. Some of these results have recently been published⁴.

THE OCCOQUAN EXPERIENCE

In December of 1975, a contractor to EPA's Office of Water Supply reported results from the previous summer's virus sampling of the Occoquan (Va.) Reservoir System⁵. The contract laboratory had collected 45 samples from six sites throughout the system. In addition to the isolation of virus from the raw water sampling sites, viral recoveries were reported to have been made from the three finished water sites.

The seven samples that yielded virus are shown in Table 2. Sampling sites A (upstream from pollution sources) and B (downstream from pollution sources) were located on a major tributary upstream from the

reservoir. The relatively low coliform and virus counts indicated that the water was not heavily polluted with domestic waste even though site B was only about two miles below the discharge of a secondary sewage treatment plant. The bacteriological quality of the water appeared to have improved by the time the water reached site C (the plant intake), and after treatment the coliform count dropped below the detectable level. However, the virus counts were essentially the same in the positive samples from all six sites.

Table 3 shows the virus recovery data and some water parameters at the three finished water sites. The indicated parameters are considered to be important for chlorine disinfection of viruses. Site D was in the treatment plant and sites E and F were in the distribution system several miles from the plant. The water parameter measurements at all three sites (a free chlorine residual above 1.0 mg/l, a pH near neutrality and a turbidity approaching 0.1 NTU) are generally considered to be excellent conditions for viral disinfection. The reported finding of 11 poliovirus plaques in a total of 1560 liters (410 gal) of finished water having the characteristics noted above is unprecedented and, if confirmed, could have far reaching implications for water treatment practice.

A CONTRARY VIEW

Several conditions and observations have been noted that are at odds with a positive virus finding in this finished water. These are summarized in Table 4. The source water supply for the Occoquan treatment system is the reservoir produced by the impoundment of Occoquan Creek. It is approximately 16 miles long and contains about 60 million m³ of water - typically a six-month supply for the water treatment system. A major tributary receives secondary effluents from 12 small sewage plants at points several miles from the reservoir. No point source waste water flows directly into the reservoir. However, two parks with septic fields are located on the shoreline about midway along the length of the reservoir and some farm houses are also located on the watershed. Bacteriological tests suggest that, from a microbial standpoint, the reservoir is a satisfactory water source and is considerably better than many surface water supplies used in the U.S. On the seven days between early June and late August that the source water was sampled, coliform counts ranged from an MPN of only 8 to 350/100 ml and the fecal coliform count ranged from < 2 to 13/100 ml. On the 4 days that viruses were recovered from the finished water samples, water during the prechlorination step had a pH of 5.7 to 6.4 and a low turbidity (2.3 to 3.4) - good conditions for an intermediate disinfection step. Likewise, chlorine in the finished water was present in concentrations and under conditions that would allow virus inactivation. Laboratory studies with enteric viruses have indicated that viral inactivation of six logs and greater could be expected under such treatment conditions^{6,7}.

The primary source of human enteric viruses in surface waters is domestic sewage. When this sewage represents the combined waste of a community, several of the 100 known enteric virus types are usually isolated from the contaminated water. Since all 21 isolates from this study were poliovirus 1, the possibility of exogenous contamination of these samples must be considered.

In addition, the sampling of the Occoquan finished water under a separate program has not resulted in isolation of a single virus from

samples collected biweekly since November 1974. And finally, the authors' failure to find viruses in another finished water subjected to similar treatment of a much more polluted source water must be considered in the evaluation of the Occoquan data.

On the other hand, a close scrutiny of treatment procedures and plant design revealed that less than optimum disinfection conditions existed during the sampling periods. These deficiencies relate primarily to filter backwash procedures, and floc carryover and breakthrough. However, in our opinion, these deficiencies only tended to lower the amount of virus over-kill that existed in the treatment procedure and in no way provided conditions that would allow virus penetration at this plant if current viral disinfection theory is correct.

Were these 11 poliovirus units present in the finished water of the Occoquan System? There is no consensus at the present time. EPA and the contractor are currently involved in an extensive effort to obtain further data so that a more informed opinion may be made as to the source of the isolated poliovirus.

OTHER ASPECTS

The authors would now like to touch on two other aspects of the Occoquan experience - one of a technical nature and one of public relations. There has been very little mass media or consumer response to this finding. This is surprising since the area served by this plant includes bedroom communities of Washington, D.C. A press release authorized by local authorities having an interest in the finding was picked up by only one local newspaper. A short, non-sensational article appeared off-front-page on the day following the release. A more extensive release by EPA a few days later was not picked up (to the authors' knowledge) by a single newspaper or TV station. Since that time in early 1976, two related articles have appeared in local newspapers. However, these articles seemed to be aimed at the political controversy over the general water-system plans for the area rather than the findings of this study. One might conclude from this response that a straight-forward and forthright press release is the way to prevent the alarm and sensationalism that could result from such studies - studies that are designed to evaluate the environmental health risk.

The technical question that the authors would like to respond to is this: what controls are needed to prevent contamination of samples tested in studies like these and indeed what controls are needed to prevent the question from even being raised? The authors' concern is that with the publication of a standardized virus recovery procedure and with the pressure and interest to conduct virus monitoring, the question will be raised again and again over reported positive virus findings. The threat of contamination looms over all work of this type. Most of the samples collected may be expected to be negative for virus. Positive virus results may occur only sporadically and, even then, only low numbers of virus may be isolated. Therefore, we must be sure that no exogenous virus has entered the water sample or the virus isolation system.

OTHER EXPERIENCES

The authors are aware of a few occasions where investigators have wrestled with the significance of virus isolations in their own

lab. In 1972, it was reported that viruses (three human serotypes) had been isolated from finished water samples collected from two New England communities⁸. This finding was reported at a congressional hearing, and when picked up by the press caused a great deal of public concern. Unreported was the investigator's own questioning of the reliability of his data. In conversations with the investigator one of the authors became well aware of his frustration and anxiety over what precautions and control systems should be instituted to remove the doubts that obscured the significance of his findings. Ultimately, the results were officially determined to be invalid. However, some people involved with this work remain convinced that viruses were present in these finished waters.

In another EPA laboratory in 1972, an adenovirus was isolated from a finished water sample collected in a southern state. This same virus type had been grown in that laboratory to high titers a few weeks prior to the virus isolation. Considering the facts that (1) our isolation facilities were poor at that time, (2) a common freezer had been used for storage, and (3) an additional 24 samples from the same site had been negative for virus, we judged the isolate to be a laboratory contaminant, even though no direct contamination route was apparent.

Recently an investigator in a private laboratory related a contamination experience in his lab. A surface water sample from a stream that had previously been shown to be negative for virus yielded high virus counts. The sample had been processed by a virus concentration procedure that included an aluminum-hydroxide-flocculation step followed by centrifugation of the sample in a continuous-flow centrifuge. The isolates were subjected to neutralization tests and found to be of the same virus type as the seed virus (positive control) used to test the recovery procedure on the previous day.

Subsequent tests revealed that the seed virus was able to survive the equipment clean-up procedure. This procedure included the soaking of the centrifuge components in a quaternary ammonium disinfectant at the recommended concentration for 24 hours followed by a one-hour hot water and detergent soak. The components were then rinsed in tap and distilled water and allowed to air dry. A subsequent rinse of the centrifuge tubing with 5 ml of saline showed that viable seed virus could be recovered from the tubing inner wall and could therefore contaminate the next sample processed through the equipment.

This investigator has satisfactorily demonstrated a weak link in his contamination control procedure for the isolation of virus from water and has corrected his protocol accordingly. However, he is very concerned over the possibility of exogenous contamination of some aerosol samples that have yielded virus of the same type as the seed virus. Even though he has been unable to uncover a contamination source, the lack of confidence in the reliability of his data remains.

NEED FOR CONTROLS

The possibility of contamination of environmental samples with seed virus is a concern of many investigators. The addition of known amounts of a seed virus is a vital procedure for determining the efficiency and sensitivity of a virus recovery system. Most investigators prefer to conduct such studies with water from each system that is sampled. Therefore, the recovery of a single virus type that

is identical to the seed virus tends to raise a question of contamination. To overcome this problem, a seed virus is needed that contains a marker that is not present in naturally occurring virus of the same type. Unfortunately, a satisfactory seed virus candidate with such a marker is not presently available.

It is important to note that the examples mentioned above are from the laboratories of experienced environmental virologists and not from novices to this research area. It is clear from these few examples that strict control procedures are required to ensure the authenticity of virus isolations in this sensitive area. Table 5 shows what we believe are minimum control requirements for conducting these studies.

The shedding of viruses by infected workers is a potential source of contamination for a water sample. In order to determine the likelihood of this occurrence, throat and rectal swabs should be routinely collected from all workers involved with sample collecting and processing. These swabs can then be tested for viruses only if viruses are isolated from the water sample thereby providing a meaningful control at a minimum cost and effort.

The contamination of samples may also occur during their frozen storage. The expansion of fluid or the contraction of the air within a container (especially plastics) can cause it to break or crack and thereby release a portion of its contents. The release of extremely small volumes of a high-titered virus material can contaminate the freezer and thus provide conditions for a frozen water-sample container to be contaminated. Further processing may permit the entry of exogenous virus into the sample. Therefore, the common freezer storage of water samples with virus preparations or other environmental samples known or suspected to contain high numbers of viruses is not advised.

The confirmation of each isolate as a virus is also considered to be a required control procedure. Even though it is not a contamination control, it is none-the-less a control on the accuracy of the interpretation of the results. The appearance of cytopathic effect (CPE) or plaques in a cell system inoculated with a water sample is not sufficient evidence to conclude that a virus is present. The experiences of careful investigators have been that many of these cell changes can be produced by bacteria, protozoa and ill-defined toxic materials. Isolates that fail to yield CPE on subsequent passage in cell culture should not be considered to be viruses. All isolates must be verified as viruses and if possible identified as a serotype.

Data are insufficient at the present time to determine the absolute control requirements needed. However, the authors are hopeful that this report will stimulate interest in the subject and that workers in the field will begin to share their findings and beliefs to the end that adequate controls can be included in these studies and environmental virology efforts will not be continually plagued by the questioning of virus isolation data.

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TABLE 1

BASES FOR CONCERN THAT VIRAL DISEASE MAY BE
TRANSMITTED BY DRINKING WATER

1. Human enteric viruses can survive for extended periods in the aquatic environment and these viruses have been readily isolated from wastewater and polluted surface water used as source water for treatment plants.
2. Lab data have shown most enteric viruses to be more resistant than indicator bacteria to inactivation by water disinfectants.
3. Infectivity tests have shown infection could be caused by one poliovirus unit.
4. A consistently high endemic level of infectious hepatitis occurs in the U.S. and investigations have shown contaminated water to be a source of IH outbreaks.
5. Sporadic outbreaks of non-bacterial gastroenteritis suspected of being waterborne occur rather frequently and an endemic level of gastroenteritis occurs in the United States; waterborne viruses may possibly be responsible.
6. Increased water demand has made direct recycling of wastewater almost a reality for some water systems and conventional water treatment was not designed to produce potable water from wastewater.

TABLE 2

REPORTED VIRUS ISOLATIONS FROM THE
OCCOQUAN RESERVOIR SYSTEM DURING THE 1975 STUDY

Site	Date	Virus Recovered (PFU)	Sample Volume (liters)	Total Coliform per 100 ml
Tributary (A)	8/19/75	2	372	8,000
Tributary (B)	6/4/75	8	323	7,000
Intake (C)	7/8/75	2	380	130
Finished Water (D)	7/23/75	3	399	0
Finished Water (D)	8/18/75	3	399	0
Finished Water (E)	6/5/75	4	380	0
Finished Water (F)	6/3/75	1	380	0

TABLE 3

REPORTED VIRUS ISOLATIONS AND WATER CHARACTERISTICS
FROM THE THREE FINISHED WATER SITES

Site	No. Virus Samples Pos./Collected	PFU Recovered	pH	Turbidity	Chlorine Residual (Free)
Tmt. plant (D)	2/6	6	7.4	0.18	1.5
Dist. System (E)	1/3	1	7.5	0.13	1.3
Dist. System (F)	1/3	4	7.7	0.12	1.3

TABLE 4

CONDITIONS AND OBSERVATIONS THAT TEND TO REFUTE
THE PRESENCE OF HUMAN VIRUSES IN THE FINISHED
WATER OF THE OCCOQUAN SYSTEM

1. Coliform and viral analyses of the source water at the plant intake did not show elevated counts that would be indicative of recent domestic pollution.
2. Water treatment included prechlorination at a level of ≥ 2 mg/l for 4 to 5 hours under conditions considered desirable for good intermediate disinfection.
3. Finished water at the time of the positive virus findings had a free chlorine residual ≥ 1.3 mg/l.
4. All 23 isolates from the six sites were of the same serotype - poliovirus 1.
5. Ten 1900-liter (500 gal) finished water samples collected by EPA from a plant whose source water had been found to contain up to 85 viral units/100 gal and fecal coliforms up to 60,000 colonies/100 ml have all been negative for viruses.

TABLE 5

CONTROL REQUIREMENTS FOR FINISHED
WATER VIRUS SAMPLING

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1. Personnel directly involved in sample collecting and handling should routinely have throat and rectal swabs collected. They should be processed if a virus-positive water sample is found.
 2. Aseptic technique and a closed system should be used for sample collecting and processing.
 3. When samples are to be stored prior to testing, they should be placed in ultralow temperature freezers that contain no other type of virus sample.
 4. Samples should be processed in isolation facilities where no other type of virus sample is handled.
 5. Multiple barriers to air contamination should exist, i.e., separate isolation facility, laminar flow hoods, etc.
 6. All isolates must be confirmed as being viral.
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